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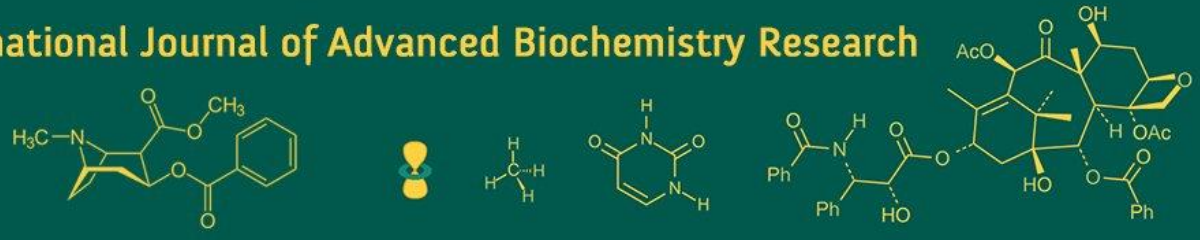

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Combine effect of oregano essential oil and citric acid on the quality of salted dried giant catfish (*Arius thalassinus)* during ambient storage condition: Biochemical and microbial attributes

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Abstract
The combined effect of oregano essential oil and citric acid on the shelf life and quality of salted dried Giant catfish (*Arius thalassinus) was* studied at ambient storage conditions. Quality assessment was based on the determination of biochemical and microbiological indices. The Trimethylamine Nitrogen, Total Volatile Basic Nitrogen, Peroxide value And Total Plate Count were higher in the control fish sample. The fish sample treated with combined oregano essential oil 0.2% (v/w) and citric acid 1%(w/w) has a lower value of Trimethylamine Nitrogen, Total Volatile Basic Nitrogen, Peroxide value, Total Plate Count and Total Fungal Count which suggests the strong antioxidant activity of oregano essential oil and the antimicrobial activity of citric acid. The combined treated sample of combined oregano essential oil 0.2% (v/w) and citric acid 1%(w/w) has a shelf life up to 90 days while sample treated with combined oregano essential oil 0.2% (v/w) and citric acid 1%(w/w) alone has shelf life up to 75 days and control sample has shelf life up to 60 days. The oregano oil and citric acid in the samples slower process of fish spoilage and maintain the quality attributes of the fish sample.

Keywords: *Arius thalasinus,* Ambient storage, Citric acid, Drying, Salting, Oregano essential oil

Introduction
Seafood is a valuable source of proteins, important fatty acids, minerals and vitamins to humans. In some developing countries they represent the main protein source in nutrition. They are good sources of important nutrients and constitute desirable components of a healthy diet. The high nutritional value and easy digestibility are the advantages of fish as food. Fishes are rich sources of omega-3 (n-3) long-chain polyunsaturated fatty acids (PUFAs).

Fresh fish and other seafood products are particularly vulnerable to deterioration due to enzymatic activity and post-mortem microbial development. This deterioration is a result of inappropriate handling during storage, distribution and marketing as well as a lack of refrigeration. The increasing demand for high quality fresh seafood has developed the methods and technologies for better fish preservation (Anderson & Anderson, 1991) [4].

There are various traditional methods available for the preservation and processing of fish for consumption and storage. These include smoking, drying, salting, fermentation and various combination of these. Among them, drying is an ancient preservation method widely employed by humans and it applies to various foods. The fundamental principle of drying involves removing the moisture from the food, resulting in reduced mass and volume, thereby facilitating transportation, storage, packaging, and offering economic benefits. Additionally, the primary objective of this preservation technique is to lower water activity through elevated temperatures, which inhibits microbial activity and extends the product's shelf life (Ratti, 2001) [44].

Artificial drying methods offer several advantages compared to natural drying methods. Okoro & Madueme, (2004) [38] reported that artificial methods are highly efficient in removing large amounts of moisture from the product. Moreover, these methods allow for the precise control of various factors, including temperature, drying air flux, and drying time.

Salting plays a crucial role in the drying process and it serves as an essential preliminary step to achieve a commercially viable product with excellent shelf life and quality. The addition of salt before the drying enhances the drying process by rapidly reducing water activity to optimal levels.

Oregano essential oil (OEO), derived from *Origanum vulgare L*., is widely recognized for its antibacterial and antioxidant properties among a wide range of essential oils (Eos) (Hosseini *et al*., 2013b) [25]. These actions are primarily attributed to the two phenols carvacrol and thymol, which make up a large portion of oregano essential oil, as well as the monoterpene hydrocarbons p-cymene and γ-terpinene, which are present in smaller amounts (Baydar *et al*., 2004) [8]. Carvacrol was shown to be an effective antibacterial agent and is a significant component of oregano essential oil (trace levels account for 82%) (Burt, 2004) [10]. thymol (trace amounts to 64%) has received considerable attention as an antimicrobial agent showing very high antifungal activity, being also an excellent food antioxidant (Sanchez-Garcia *et al*., 2008) [47].

The *Arius thalassinus* is predominantly a brackish water demersal species, which is also found in a depth range of between 10 to 195 meters (Fischer & Bianchi, 1984) [16]. *Arius thalassinus* forms a major part of the catfish catches landed by trawlers in Veraval and Mangrol. Most of the landed catfish have a very good demand in the domestic market and export markets both as frozen or dried.

Recent research has been focusing on substituting synthetic antioxidants with natural alternatives to potentially provide nutritional and therapeutic benefits (Frankel, 1995; Decker, 1998) [19, 14]. Citric acid (CA) and its salts are well-known for their ability to act as chelators and acidulants in biological systems. They also serve as synergistic agents for primary antioxidants, demonstrating favourable effects on fish oil and emulsions (Kelleher *et al*., 1992; Osborn-Barnes & Akoh, 2003) [30, 39], minced fish (Hwang & Regenstein, 1988; Stodolnik *et al*., 1992) [27, 50], as well as fish fillets (Badii & Howell, 2002; Aubourg *et al*., 2004) [7, 6]. The main objective of the present work is to see the combined effect of salting, oregano essential oil and citric acid on the shelf life and quality retention of fish at ambient storage with microbial and biochemical indices determination.

Material and Methods

Fish sample preparation

Fresh giant catfish (*Arius thalassinus*) measuring 48.03 ±0.06 cm an average length and average weight of 960.66±0.45 gm were caught off the west coast of Gujrat using a trawl net. It was transported in an insulated box in iced condition (0-2°C) from the Veraval fish landing center to the Fish Processing laboratory at the College of Fisheries Science, KU, Veraval.

Fish was washed, cleaned and dressed using a knife to remove the head, fins, scales, gill and gut followed by washing with potable fresh water. During processing, the dangerous and sharp dorsal and pectoral spines were carefully removed from the catfish using knives. The fish were then manually gutted, removing the head, fins, gills, and entrails. To eliminate blood and other impurities, the gutted fish were washed with chilled running water. Then divided in to four equal lots and give different treatments.

Treatment of fish sample

In treatment T_0(C), the fish samples were mixed with salt at 3:1 (fish: salt) in a plastic tray (Al Ghabshi *et al*., 2012) [3]. In treatment T_1, the fish sample was mixed with salt at a ratio of 3: 1 (fish: salt) and oregano essential oil was added at 0.2% (v/w). In treatment T_2, the fish sample was mixed with salt at a ratio of 3:1 (fish: salt) and citric acid powder was added at 1% (w/w). In treatment T_3, the fish sample was mixed with salt at a ratio of 3:1 (fish: salt) and added both oregano essential oil at 0.2% (v/w) and citric acid powder at 1%(w/w) were added. All samples were left to rest for 24 hours to ensure proper penetration of the treatment. After the application of a treatment, the samples were dried in an artificial dryer in which the temperature of the dryer was maintained at 45^0 C for 48 hours to get a moisture content of less than 30% for semi-drying of fish. The dried giant catfish (*Arius thalassinus*) were packed individually in Low density polyethylene plastic bag LDPE (200 gauge) for better storage of dried fish. All the packed samples were kept at ambient temperature and stored in a plastic box for analysis. Sampling was carried out at every 15 days intervals.

Proximate analysis

Moisture, crude protein, crude fat, and ash contents of the fish sample were determined using standard AOAC (2006) methods, including hot air oven, micro Kjeldahl, and Soxhlet extraction techniques.

Biochemical Analysis

PH in the fish sample was determined by the pH meter method described by AOAC (2006). TMA-N and TVB- N were determined as trimethylamine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N) by the micro diffusion method described by Beatty and Gibbons (1937) [9]. TMA-N and TVB-N were calculated and expressed mg/100g of the samples. The peroxide value (PV) of the fish sample was determined from the lipid extract iodometrically method according to Jacobs (1958). The peroxide value (PV) of the fish sample was expressed as miliequivalent peroxide/kg of lipid.

Microbiological Analysis

The microbiological characteristics of the fish sample were determined according to the standard method recommended by AOAC (2006). Plate Count Agar (PCA) was used for the enumeration of the total plate count. The Total Fungal Count (TFC) of the giant catfish sample was determined by the spread recommended by AOAC (2006). Potato dextrose agar was used for the enumeration of the total fungal count. Colonies were counted and the results in colonies per g.

Statistical Analysis

Statistical analysis was based on triplicate analysis for each sample at each specific storage time. ANOVA (Analysis of variance) statistical technique was used to find out the significant difference in samples between the treatments as per the standard statistical methods. (Snedecor & Cochran, 2014) [18]. The analysis of variance (ANOVA) was carried out based on the experimental data using IBM BASIC Windows release 1.13 to know the significant difference between different treatment combinations and to find the best treatment combination.

Result and Discussion

Proximate composition of Raw material

The moisture content of raw material was 78.11±0.40, crude protein content was 17.03±0.15, crude fat content was 2.35±0.06 and ash content was 1.38±0.02 (mean±SD). The proximate composition of these species compares well with the result obtained by Abraha *et al*. (2017) [2] and by Kristanti *et al*. (2023) [31]. The slight variation in the proximate composition of raw material was due to the environmental conditions, type of feed and difference in sex, weight, size and season.

Biochemical changes

Change in pH during ambient storage

The acidity (pH) of fish increases as it spoils due to the action of certain microbes that break down carbohydrates and produce acidic byproducts. The pH value is a reliable indicator of the degree of freshness or spoilage. The changes in the pH of dried giant catfish (*Arius thalassinus*) during ambient storage conditions are shown in Figure1. The pH valu of fresh giant catfish (*Arius thalassinus*) was 6.97±0.06 (mean±S.D) in our study. But when salt is added during salting, the pH value decreases due to the increase of the acidic compound. The initial pH value of the sample after drying was 6.33±0.06, 6.17±0.07, 6.23±0.12 and 6.13±0.09 for T_0(C), T_1, T_2, and T_3 respectively with no significant differences in all samples. After the storage, the pH value was increased to 7.57±0.06 for T_0(C), 7.17±0.06 for T_1, 7.27±0.06 T_2 and 6.97±0.15 for T_3. The highest pH value was found in the T_0 control and the lowest pH value in T_3.

The increase in pH value is due to the production of alkaline compounds such as ammonia, due to the growth of spoilage bacteria (Chaijan *et al*. 2005) [11]. Goulas *et al*., 2007 [21] found the lowest pH value when oregano essential oil (OEO) was applied with light salting and MAP in sea bream (*Sparus aurata*) during refrigerated storage conditions. Similarly, Kazemi & Rezaei (2015) [29] found the lowest pH when oregano essential oil (OEO) was applied with gelatin-algin film compared to control in rainbow trout fillet. The fish products are acceptable up to a pH of 6.8 but are considered to be spoiled above 7.0 pH (Huss, H.H. 1988). The pH range between 6.8 and 7.0 is typically seen as the borderline for acceptability (Erkan *et al*., 2011) [15].

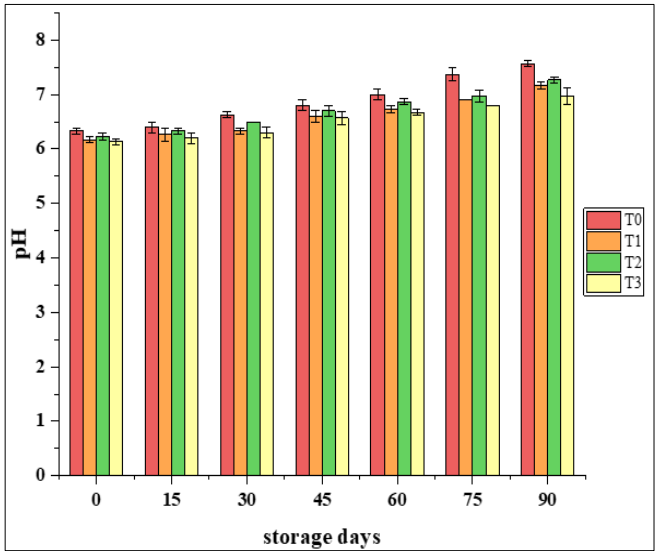

Fig 1: Change in pH content of dried giant catfish (*Arius thalassinus*) during ambient storage.

Change in Trimethylamine Nitrogen (TMA-N) during ambient storage

TMA is produced from trimethylamine oxide (TMAO) by the action of intrinsic enzymes and certainly through bacterial action. The changes in TMA-N content in all samples (T_0(c), T_1, T_2, and T_3) increased progressively with longer storage periods shown in Figure 2. The initial TMA-N content was 13.72±1.96 mg/100g for T_0 (control), 13.28±0.5 mg/100g, 13.51±0.83 mg/100g and 13.16±0.66 mg/100g for T_1, T_2 and T_3 respectively (mean±SD). After the storage, the TMA-N was increased up to 61.37±0.4 mg/100g, 53.74±0.27 mg/100g, 57.53±0.2 mg/100g and 47.49±0.27 mg/100g for T_0 (control), T_1, T_2 and T_3 respectively (mean±S.D). The highest TMA-N content was found in treatment T_0 (control) and the lowest TMA-N content was found in treatment T_3, T_1 and T_2 sequencing.

The lowest TMA-N content in other treatments compared to control is mainly due to the antimicrobial properties of oregano essential oil (OEO) and the antioxidant properties of citric acid. This is in agreement with the findings of Goulas & Kontominas (2007) [21] demonstrated that in comparison with air-packaged, MAP packed and MAP packed with salt alone, the MAP packed with salt and oregano essential oil (OEO) combined to decrease the the rate of TVB-N in the fish during refrigerated storage. Furthermore, Kusuma & Teerawut (2014) [33] noted that addition of oregano essential oil (OEO) along with alginate-based coating reduced the TMA-N content in shrimp compared to the control sample and alginate-based coated sample at refrigerated storage conditions. The acceptable limit of TMA-N was 50 mg/100g was considered as a limit for dried fish above which fish is termed as spoilage (Connell, 1995) [12]. However, control (T_0) sample was acceptable for up to 60 days, the T_1 and T_2 samples were acceptable for up to 75 days and the T_3 sample was acceptable for up to 90 days.

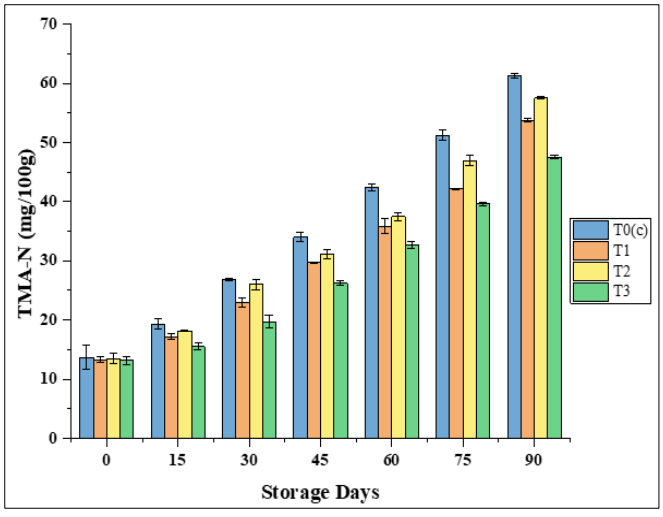

Fig 2: Change in TMA-N (mg/100g) of dried giant catfish (*Arius thalassinus*) during ambient storage.

Change in Total Volatile Basic Nitrogen (TVB-N) during ambient storage

TVB-N is one of the most frequently utilized measures for assessing fish quality. Its elevation correlates with the activity of spoilage bacteria and endogenous enzymes. The changes in TVB-N content in treatment T_0 (control), T_1, T_2 and T_3 show an increasing trend with an increased storage

period shown in fig 3. The initial TVB-N content was 42.73±0.28 mg/100g for T_0 (control), 42.66±0.19 mg/100g for T_1, 42.67±0.10 mg/100g for T_2 and 42.53±0.28 mg/100g for T_3 samples (mean±SD). After 90 days of storage at ambient temperature, the TVB-N content increased up to 222.55±1.53 mg/100g for T_0 (control), 210.81±0.89 mg/100g, 218.59±1.88 mg/100g and 194.19±0.55 mg/100g for T_1, T_2 and T_3 samples respectively (mean±SD).

The lower TVB-N content in treatments T_1, T_2 and T_3 indicated that the incorporation of oregano essential oil (OEO) and citric acid could inhibit the growth of bacteria. According to Hosseini *et al*. (2016) [24], the incorporation of oregano essential oil (OEO) with fish gelatin in rainbow trout fillet rapidly reduced bacterial population or decreased the bacteria's ability for oxidative deamination of non-protein nitrogen compounds or both, which was due to the effect of oregano oil. Furthermore, similar decreasing trends in TVB-N content were found by Zhang *et al*., (2021) [51] when citric acid is applied with rosemary extract in shrimp at chilled storage. Abd El-Fatah *et al*. (2023) [1] also found lower TVB-N values in Basa (*Pangasius bocourti*) Fillets when citric acid is applied alone or in combination with other ingredients compared to the control sample. Pyrgotou *et al*. (2010) [18] investigated the most significant inhibition of TVB-N compound formation in rainbow trout fillets subjected to treated samples with different concentrations of oregano essential oil with salting and packed in modified atmospheric packaging conditions. A TVB-N value of 200 mg/100g has been suggested as the threshold for spoilage in cured fish (Srinivasan & Joseph, 1966; Prasad & Rao, 1944). The control (T_0) sample was acceptable for up to 60 days, the T_1 and T_2 samples were acceptable for up to 75 days and the T_3 sample was acceptable for up to 90 days.

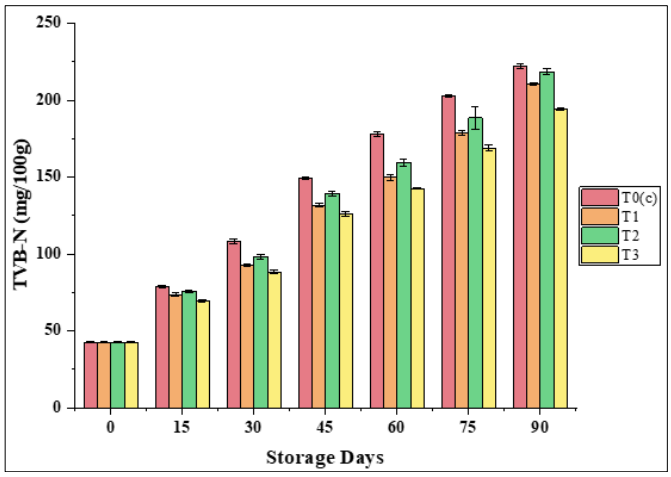

Fig 3: Change in TVB-N (mg/100g) of dried giant catfish (*Arius thalassinus*) during ambient storage.

Change in Peroxide value (PV) during ambient storage

The Peroxide value (PV) is used to measure the primary lipid oxidation, especially hydroperoxides. The changes in PV content in treatment T_0 (control), T_1, T_2 and T_3 show an increasing trend with an increased storage period shown in fig 4. The initial PV content for sample T_0 (control) was 3.73±0.07 m.equ./kg and 3.56±0.11 m.equ./kg, 3.7±0.03 m.equ./kg, 3.52±0.08 m.equ./kg for T_1, T_2 and T_3 samples respectively (mean±S.D). After storage, the PV content was increased up to 25.78±0.48 m.equ./kg for T_0 (control) and 22.18±0.38 m.equ./kg, 23.03±0.78 m.equ./kg, 19.77±0.2 m.equ./kg for T_1,T_2 and T_3 samples respectively (mean±S.D). The T_0 (control) sample had the highest PV value and the T_3 sample treated with oregano essential oil and citric acid had the lowest value.

Dried fish are susceptible to oxidation due to exposure to oxygen, leading to lipid peroxidation as the fish ages. Higher moisture levels and environmental factors like yeast, mold, and bacteria can accelerate lipid breakdown, increasing peroxide values (PV). A similar increasing trend was observed by Majumdar *et al*. (2018) [35] the initial peroxide value of dried fish *mystus vittatus* was 12.08 0.97 meq/kg of lipids and it increased continuously with storage. Nurullah *et al*. (2007) [37] also found an increasing trend of peroxide value in mola-dried fish and it increases progressively with the storage period. The lowest PV content in the treatment T_3 was mainly due to the incorporation of oregano essential oil (OEO) and citric acid which reduces microbial growth and retarded the lipid oxidation of fish. This is in agreement with the finding of Mexis *et al*. (2009) [36] demonstrated that the application of oregano essential oil (OEO) alone and combined with an oxygen absorber in rainbow trout fillet prevents lipid oxidation and reduces the peroxide value compared to the control sample. Hosseini *et al*. (2016) [24] observed similar low peroxide values when oregano essential oil was applied with fish gelatin in fish. Similarly, results also found by Pourashouri *et al*. (2009) [40] and Rostamzad *et al*. (2010) [46] who demonstrated that compared to the control sample application of citric acid alone and in combination with ascorbic acid prevents lipid oxidation in wels catfish and sturgeon fillets respectively. Furthermore, Qiu *et al*. (2016) [43] observed that the addition of citric acid with chitosan reduces lipid oxidation compared to the control sample in fish.

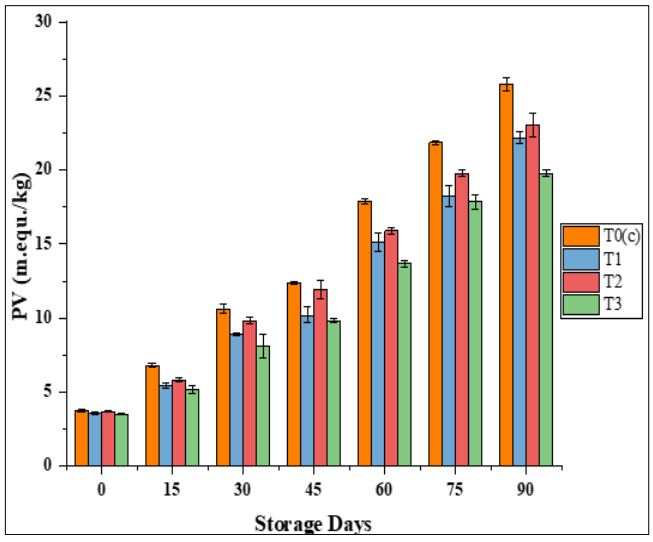

Fig 4: Change in PV (m.equ./kg) of dried giant catfish (*Arius thallasinus*) during ambient storage.

Microbial analysis

Change in Total Plate Count (TPC)

The initial total plate count was 3.57±0.05 log CFU/g for T_0 (control) and 3.42±0.04 log CFU/g, 3.46±0.10 log CFU/g and 3.29±0.06 log CFU/g for T_1, T_2 and T_3 samples respectively (mean±S.D). After 90 days of storage, the total plate count increased to 5.40±0.06 log CFU/g, 5.19±0.06 log CFU/g, 5.29±0.06 log CFU/g and 4.86±0.02 log CFU/g for T_0 (control), T_1, T_2 and T_3 respectively (mean±S.D). The T_0 (control) sample found the highest total plate count.

Similarly, increasing trends were found by Guizani *et al.* (2008) [22] salted dried Shark stored at ambient temperature. Furthermore, Cyprian *et al.* (2017) [13] also found a similar increasing trend of total plate count in dried capelin (*Mallotus villosus*) stored at ambient temperature.

The lowest bacterial count was found in the fish treated with oregano essential oil (OEO) and citric acid sample which is mainly due to the antimicrobial effects of the oregano oil phenolic components thymol and carvacrol, known to exert antimicrobial activity and antioxidant properties of citric acid (Burt 2004; Lambert *et al.* 2001; Holley & Patel 2005) [10, 34, 23]. The addition of different concentrations of oregano essential oil (OEO) in swordfish fillets reduces the growth of microbes (Giatrakou *et al.*, 2008) [20]. Frangos *et al.* (2010) [18] observed a decrease in total plate count in rainbow trout fillets treated with different concentrations of oregano essential oil (OEO) with salting and packed in vacuum packaging compared to the air-packed sample alone. A similar lower bacterial count was also found by Pyrgotou *et al.* (2010) [18] when they added oregano essential oil (OEO) and salt to rainbow trout fillets and packed them in MAP conditions compared to control samples. Zhang *et al.*, (2021) [51] observed that the combination of citric acid with rosemary extracts significantly preservative effect by maintaining low bacteriological samples in shrimp.

The maximum permissible microbiological load in salted dried fish, as per the FSSAI, is 5 log CFU/g. (FSSAI, 2017). In the current study, the T_0 (control) sample is acceptable for up to 60 days. T_1 and T_2 samples were acceptable for up to 75 days. The T_3 sample is acceptable p to 90 days of storage which indicates that the treatment effect inhibits microbial growth.

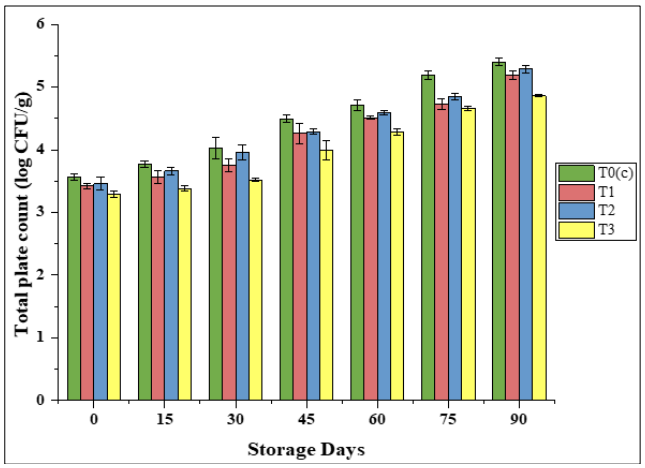

Fig 5: Change in Total plate count (log CFU/g) of dried giant catfish (*Arius thallasinus*) during ambient storage.

Change in Total Fungal Count (TFC)

Initially, the total fungal count in the sample was not observed. The interaction effect of treatments and storage period was found significant. After the storage period of 60 days total fungal count was observed in T_0 (control) but there was an absence of total fungal count in T_1, T_2 and T_3 samples. After 75 days total fungal count was observed in the T_1, T_2 and T_3 samples. The total fungal count is 4.34±0.1 log CFU/g for T_0 (control) and 2.95±0.18 log CFU/g, 3.18±0.07 log CFU/g and 2.58±0.17 log CFU/g for T_1, T_2 and T_3 respectively (mean±S.D). At the end of storage, the highest fungus was found in the T_0 (control) sample and the lowest fungus was found in the T_3 sample.

The growth of the fungus is mainly due to increased water activity and moisture content in the samples (Kumar *et al.* 2013) [32]. Similar fungal growth was found by Guizani *et al.* (2008) [22] in salted dried sharks. The lowest total fungal count in the sample is mainly due to the antimicrobial effects of the oregano oil phenolic components thymol and carvacrol, known to exert antimicrobial activity and antioxidant properties of citric acid. According to FSSAI (2017), the maximum mould limit in salted dried fishery products is 500 cfu/g (2.7 log cfu/g).

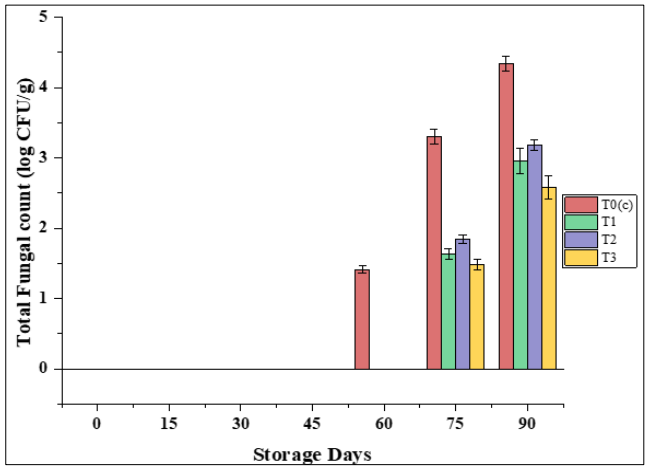

Fig 6: Effect of treatments on total fungal count progression in samples during 90 days storage period.

Conclusion

The results from the current study suggest that salting, oregano essential oil and citric acid-treated dried samples have prime quality and safety when packed in LDPE pouch packaging. The natural or synthetic preservatives significantly impact on the biochemical, microbiological characteristics of giant catfish samples.

The combined application of oregano essential oil and citric acid significantly extended the shelf life of the product up to 90 days under ambient storage conditions. In comparison, treatments with oregano essential oil or citric acid alone prolonged the shelf life to 75 days, whereas the control sample remained stable only up to 60 days. The preservatives exhibit good antimicrobial and antioxidant properties, decreased growth of bacteria and oxidation of fat and protein. The use of essential oils, for instance oregano essential oil, and antioxidants, like citric acid, in salting and drying reduces the rate of spoilage under ambient storage conditions. This not only maintains product quality but also improves the drying technology, enhance its economic viability in both domiciliary and overseas markets.

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